ANATOMICAL STUDIES IN THE GENUS GENTIANA TOURN

by

CHRISTINE M. CANT, B.Sc.

Ph.D. Thesis.

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C O N T E N T S.

Introduction.

The genus Gentiana, composed of over eight hundred species is world-wide in its distribution members being found in Europe, Asia, Africa, Australia, New Zealand and North and South America. The continent of Asia seems to be the centre of distribution as here the greatest number of species is found, the majority being described from Western China and the adjoining states. As a genus it is by no means confined to any one habitat as species may be found on high alps, on screes, in woodland, in meadows, in marshes and even in peat bogs. As the flowers show considerable variation in colour and as many of the species are highly suitable for cultivation the genus has become one of horticultural importance.

While the genus has proved a fairly popular subject with systematists there has been very little investigation on its anatomical aspect. The most extensive work has been by E.Perrot in his "Anatomie comparée des Gentianacées" (1). As in this work he has been investigating the anatomy of the family as a whole, he has not given much detail on the anatomy of the genus Gentiana, especially on their roots and leaves, and therefore it is these structures which have formed the basis for this investigation.

The species investigated were obtained from plants growing in the Royal Botanic Garden,

Edinburgh and only those were studied where the specific name had been definitely confirmed. These species were:-

G.acaulis. Linn
G.Andrewsii. Griseb.
G.angulosa. Bieb.
G.angustifolia. Vill
G.asclepiadea. Linn
G.bellidifolia. Hook.f.
G.cachemirica. Decne
G.cephalantha. Franch.
G.corymbifera. T.Kirk.
G.crassicaulis. Duthie.
G.cruciata. Linn.
G.dahurica. Fisch.
G.decumbens. Linn
G.depressa. D.Don.
G.Farreri. Balf.f.
G.Fetisowi. Regel & Winkler.
G.gilvostriata.Marquand.
G.gracilipes. Turrill
G.Grombczewskii.Kusnezow.
G.hexaphylla. Maxim.
G.hexaphylla x Farreri.
G.Kochiana.Perr.& Song.
G.Kurroo. Royle.
G.lagodechiana.Kusnezow.
G.Loderi. Hook.f.
G. X Macaulayi.
G.macrophylla. Pall.
G.Makinoi. Kusnezow.
G.Newberryi. A.Gray.
G.ornata. Wall.
G.Parryi. Engelm.
G.phlogifolia. Schott & Kotschy.
G.Pneumonanthe. Linn.
G.prolata. Balf.f.
G.purpurea. Linn.
G.saxosa. Forst.f.
G.scabra. Bunge.var. Buergeri. Maxim.
G.sceptrum. Griseb.
G.septemfida. Pall.
G.setigera. A.Gray
G.sino-ornata. Balf.f.
G.stragulata. Balf.f. & Forrest.
G.straminea. Maxim.
G.tianschanica. Rupr.
G.trichotoma. Kusnezow.
G.Veitchiorum. Hemsl.
G.verna. Linn.

Method.

Roots and leaves taken from several individuals of each of the species were preserved, the roots in chromacetic acid, and the leaves in acetic alcohol. The acetic alcohol was found useful in extracting the chlorophyll thus rendering the structure in the leaf tissues more clearly visible. The sectioning was done by microtome. Three combinations of stains were used. Gentian Violet and Bismarck Brown proved the most useful generally, but Crystal Violet and Erythrosin were very good for demonstrating suberised tissues, while Gentian Violet and Iodine Green brought out the structure of the endodermis.

The first part of the investigation deals with the roots.

Gentiana - The Root.

The Young Root.

In the young root .....

The Young Root.

In the young root the early development of the tissues can be seen (G.gilvostriata Fig.1). As would be expected the outer tissues are differentiating more rapidly than those which will eventually give rise to the stele. At this stage every cell is very rich in proto-plasmic contents but only the cells in the outer tissues possess clearly defined cell walls.

The root is bounded on the exterior by an epidermis, one cell thick whose cells in cross-section appear rather columnar in shape, with their outer walls slightly thickened. In many of the cells the nucleus is clearly visible. The layer immediately inside the epidermis, also one cell in thickness, is the hypodermis. The cells composing this layer are of quite a different shape, showing in most cases a distinct tendency to assume a hexagonal outline. These cells also are rich in protoplasm with conspicuous nuclei. Of the cortex, so far, a double layer of cells has been differentiated, the outer cells resembling the hypodermis in shape while the inner that is, these more recently formed, tend to be four or five sided with a distinct tangential elongation. These cells likewise are rich in protoplasm and possess clear nuclei. The remaining cells, those occupying the centre of the root and which will eventually give rise to the stelar tissues are as yet undifferentiated.

In most of the species the development is quite normal and results in a diarch stele, the young stage showing two single xylem vessels. (G.angustifolia Fig.2). From this condition development usually results in the differentiation of a small plate of xylem. This xylem plate may be straight (G.bellidifolia Fig.3) or slightly irregular. (G. asclepiadea Fig.4). In some cases the vessels may appear in two groups.(G.andrewsii Fig.5).

While the normal condition found in the roots is diarch there are a few exceptions in the species examined. Seven species, namely:- G.acaulis (Fig.6), G.corymbifera (Fig.7), G.Fetisowi (Fig.8), G.ornata (Fig.9), G.trichotoma, (Fig.10), G.asclepiadea (Fig.11) and G.sceptrum (Fig.12) have triarch steles but this occurrence can hardly be regarded as a critical feature as G.asclepiadea may also have a diarch stele (Fig.4), while in G.sceptrum (Figs.13, 14) diarch and tetrarch conditions may be found.

In the normal diarch root the protophloem can easily be identified lying in two groups in a plane at right angles to that of the xylem plate (G.verna Fig.15). The phloem groups are usually separated from the xylem by a little parenchyme. G.Kurroo (Fig.16). The phloem cells are the smallest in the stele and are of a rather angular shape;

they are also rich in protoplasmic contents.

When the stele is triarch or tetrarch the number of protophloem groups also increases to three or four to correspond with the groups of xylem vessels (G.asclepiadea Fig.11, G.sceptrum Figs.12,14).

Surrounding the vascular tissue is the pericycle which is invariably one cell thick and is very easily recognised in all the roots (G.hexaphylla Fig. 17). The cells forming the pericycle are large and roughly circular in shape but they do not have such a large amount of protoplasm as is seen in the phloem.

External to the pericycle lies the endodermis. This is also an exceptionally well-defined and therefore easily recognisable tissue in all the roots.(G.hexaphylla Fig.17). The cells of the endodermis do not vary to any marked degree in shape as they are all, roughly oval, but they differ often in orientation as in some cells the long axis of the oval is tangential with respect to the axis of the stele while in others it is definitely radial. On the radial walls of the cells can be seen the thickening typical of endodermal cells. In the most obvious examples it takes the form of two little thickened circular areas appearing where the radial walls of two adjacent cells are in contact.(G.sceptrum Fig.18. G.acaulis Fig.19). The endodermal cells are always in close contact and thus no air

spaces appear between them. Indeed none of the tissues internal to the endodermis possess intercellular spaces.

The cortex is a very uniform tissue in construction, two or three cells in breadth, these cells being large, thin walled and possessing a rather scanty amount of protoplasm. Their shape varies and the outline may be round, irregular, or almost square. Because of their shape they are loosely packed and a considerable number of air-spaces are to be found among them (G.Kurroo.Fig.16).

An interesting feature in the cortex of many of the species is the presence of a fungus. (G.acaulis.Fig.20, G.Kurroo Fig.16). The hyphae of the fungus appear to be continuous from cell to cell passing through the wall. Apparently they do not have any harmful effect as no change seems to take place in the cell, its nucleus remaining visible and therefore indicating that the cell is still alive.

The species could be divided into three groups depending on the absence or presence of the fungus.

Group A.	Fungus absent.
G.Andrewsii	G.Kochiana
G.angustifolia	G.Newberryi
G.bellidifolia	G.ornata
G.cachemirica	G.Parryi
G.cephalantha	G.prolata
G.crassicaulis	G.setigera

G.cruciata
G.dahurica
G.Fetisowi
G.hexaphylla X Farreri
G.sino-ornata
G.tianschanica
G.trichotoma

Group B.	Fungus present.
G.acaulis	G.Loderi
G.asclepiadea	G.macrophylla
G.corymbifera	G.Makinoi
G.decumbens	G.phlogifolia
G.Farreri	G.Pneumonanthe
G.gilvostriata	G.purpurea
G.gracilipes	G.saxosa
G.Grombczewskii	G.scabra var.Buergeri
G.hexaphylla	G.septemfida
G.Kurroo	G.stragulata
G.lagodechiana	

Group C.	Fungus absent or present.
G.angulosa	G.sceptrum
G.depressa	G.straminea
G.X Macaulayi	G.verna.

From the presence of Group C it would seem that the fungus is not necessary to the roots but that when it is present it does not harm the tissue. In the majority of the species the fungus seems to be practically confined to the cortex although in one or two isolated examples hyphae can also be seen in the

epidermis (G.Farreri Fig.21).

It can be seen from the lists that as one would expect, the fungus is present in G.Farreri, absent in G.sino-ornata, and either present or absent in their hybrid G.X Macaulayi. In view of the indiscriminate manner in which the fungus seems to be present or absent it is interesting to note that it is present in G.hexaphylla and G.Farreri but is absent in their hybrid G.hexaphylla x Farreri.

Returning to the normal tissues, the cortex is bounded by the hypodermis. This tissue was found to be a constant feature throughout all the species examined. The cells of the hypodermis are usually smaller than those of the cortex and have slightly thicker walls. (G.Kurroo Fig.16, G.bellidifolia Fig. 3). They contain a fair amount of protoplasm, more than is present in the cortical cells. As the epidermis is shed the hypodermis sooner or later becomes the limiting tissue of the root.

At the young stage the root is normally enclosed by the epidermis. It differentiates early and at first its cells are almost columnar in shape and very rich in protoplasm. Occasionally the cells remain rather columnar but usually they tend to become more isodiametric. (G.hexaphylla X Farreri Fig.22. G.macaulayi Fig.23). The epidermis is essentially a tissue of the young root and rarely

persists in good condition once secondary growth has commenced. In some cases the disintegration begins very early and occurs at a stage when only two xylem vessels have been developed in the stele (G.cephalantha Fig.23) or it may even be completed by that stage (G.angustifolia Fig.2). Or again, the disintegration takes place slightly later when the xylem has formed a plate of four or five vessels,(G.bellidifolia Fig.25, G.verna Fig.14) or as will be seen later it may persist until a comparatively late stage in the growth of the root.

A feature of the roots is the entire absence of root hairs, these not being found in any of the species under examination. However in very young roots of some of the species certain of the epidermal cells show a distinct tendency to enlarge and project. The increased surface area thus resulting will undoubtedly afford better contact with the soil particles. These enlarged cells will therefore be performing the functions of, and so replacing the epidermal cells with their root hairs. (G.cephalantha Fig.26).

After the xylem plate or little groups of vessels have been developed the stelar tissues increase in amount (G.straminea Fig.27). With this increase the metaxylem is laid down, development being centri-petal as is normal in roots (G.Newberryi Fig. 28).

Almost concurrently with the development of the metaxylem the cells of the endodermis commence to divide. The first sign of endodermal division is seen in the appearance of a radial straight wall dividing each cell in two (G.Pneumonanthe Fig.29). The process then continues and additional walls appear cutting the original cell into several cells, (G.tianschanica Fig.30). 4/B

About this stage the wood cambium makes its first appearance. It arises in the tissue lying between the protophloem groups and the metaxylem (G.tianschanica Fig.30) and gradually spreads round the protoxylem groups thus forming a complete circle (G.gracilipes Fig.31)

The cells of the pericycle may also start dividing at this period of stelar activity. The division walls which appear in this region may be radial or tangential thus the pericycle may increase radially as well as on its circumference (G.tianschanica Fig30). The pericycle however may not divide until much later and often the protophloem can be recognised easily in an older specimen because of the presence of the single layered pericycle between it and the endodermis.

By this period in development many of the species have already lost the outer tissues of the root but several still have them preserved in good condition (G.tianschanica Fig.30). In G.tianschanica

there is a cortex five cells in breadth, the cells being regular and in the main octagonal in form, the latter fact accounting for the wealth of air spaces. The cortical cells are thin-walled and as can be seen in the section certain of them harbour a fungus. The hypodermis and epidermis are present and still form regular tissues, their cells being similar in size and also in shape. In the hypodermis the inner walls show less thickening than the side or outer walls while in the epidermis the outer walls are considerably thicker than the others.

The Mature Root.

The centre of the axis in the mature root is occupied by the xylem, primary and secondary, both of which tissues are in the majority of the species, quite normal (G.gilvostriata Fig.32 G.Makinoi Fig.33) but a few show a slight variation with respect to the amount of thickening on the walls of the vessels. This abnormal thickening is particularly noticeable in the secondary xylem and while it tends to occur on the tangential walls it is not invariably in that position. The result, however, is that the cavity of the vessel assumes a distinctly oval shape.(G.angustifolia Fig.34). This unusual shape of vessel is best seen in G.acaulis, G.angustifolia, G.Kochiana, G.Kurroo, G.macrophylla and G.setigera. It is not, however, a critical feature as various degrees of it

can be found between those examples given and the more normal types. It is a striking feature in these six species.

The secondary xylem does not as a rule form a completely lignified tissue. It is mixed in varying degrees with parenchyme. In some a large amount of parenchyme is present (G.purpurea Fig.35) while in others the tissue shows a much greater proportion of xylem vessels.(G.sino-ornata Fig.36). The proportion of xylem and parenchyme present is not however always constant for any one species although it does tend to be so. Thus one species may show considerable variation, in one root there being a large amount of parenchyme (G.purpurea Fig.35) while in another the amount of xylem may be much greater (G.purpurea Fig.37).

In only one of the species does the secondary xylem exist as an entirely lignified tissue. This is in G.Veitchiorum (Fig.38) where a complete ring of secondary thickening is formed. The centre of this root shows the primary xylem and surrounding it there is first of all a narrow zone where there is a little parenchyme mixed with the xylem but this parenchyme is quite absent from the next region which is composed of wholly lignified tissue. This type of secondary thickening was not found in any other of the specimens examined. The nearest approach is seen in G.prolata (Fig.40) where the xylem is plentiful but it is definitely interspersed with a little

parenchyme.

Only one abnormal feature was found in the xylem. This occurs in G.cruciata (Fig.39) where two of the xylem vessels have become blocked. The tissue round these two vessels has then become meristematic and a few new cells have therefore been formed round the blocked vessels. The walls of these cells, which border on the vessels have also become lignified with the result that the vessels have been isolated. Perrot found this taking place in G.Pneumonanthe.

The amount of secondary xylem formed seems to vary slightly with the species. In general it could be said that the secondary xylem is reasonably well developed but in some species the actual roots do not appear to attain any great size and hence in these species the xylem does not appear to progress very far with its development (G.Farreri, G.hexaphylla, G.hexaphylla X Farreri, G.ornata, and G.Pneumonanthe).

Surrounding the xylem is the cambium which gives rise to the secondary tissues (G.Makinoi.Fig.33) The cells of the cambium are quite normal and obviously actively dividing. Of the tissues to which it gives rise those resulting from its inward division have already been considered.

On its external side the cambium gives rise to a considerable quantity of tissue, which consists mainly of parenchyme. Embedded in the parenchyme

however are little patches of phloem (G.Makinoi Fig. 33). Each of these groups of phloem consists usually of from two to four sieve tubes and a similar number of companion cells (G.straminea Fig.41a). The sieve tubes are rather angular in outline usually four or five sided and only rarely can contents be seen in them, while the companion cells, distributed one to each sieve tube are considerably smaller than the latter, being about one quarter their size. In shape they are three or four sided and are quite readily distinguished as they are well supplied with protoplasmic contents. In the majority of the species, the phloem groups, that is, both sieve tubes and companion cells have slightly thicker walls than the surrounding parenchyme (G.Makinoi Fig.33)

When the size of the cells in the region of the phloem and parencyhme is taken into consideration, it is possible roughly to divide the roots of the species examined into two types. The actual species themselves cannot be thus rigidly separated by this criterion as one or two of the species possess roots of both types. With the cell size too,there is a slight difference in the thickness of the walls, the larger cells having thicker cell walls than the smaller.

In the previous paragraph it was noted that in the majority of the species the phloem groups had thicker walled cells than the parenchyme. When this

is correlated with cell size it is found that it is in the large celled type that this variation occurs (G.Makinoi Fig.33) while in the small celled type the thickening of the cell walls of the phloem is uniform with those of the parenchyme.

There is also considerable variation in the number of air spaces in this region. The tissue is normally fairly compact but occasionally it may be quite loosely packed. The shape of the cells varies with this as the looser the tissue is packed the more does the shape of the cells approach the circular (G.corymbifera Fig.42). This factor varies both in species and individual.

Mention might here be made of an abnormality found in two cases, G.asclepiadea (Fig.43) and G.purpurea (Fig.44). As in other normal instances the centre of the root is occupied by a mixture of xylem and parenchyme and in the two roots mentioned there is more parenchyme than xylem. The abnormal feature is that here and there in this parenchyme interspersed with the xylem there occur little groups of phloem tissue consisting as is usual of sieve tubes and companion cells. It is obvious that as the phloem develops in among the parenchyme cells it can only arise where there is sufficient parenchyme among the xylem to allow of its doing so. This interxylary phloem was only found in the two species quoted. Perrot seemed to find this interxylary phloem more

frequently.

The pericycle surrounds the phloem region and is of a considerable depth, the original cells having divided quite prolifically. The cells are distinctly irregular in shape due to the fact that division has taken place tangentially and radially quite indiscriminately. The cell walls also become thicker and as the cells are usually closely packed there are few or no air spaces. The size of the cells shows some correlation with the cell size in the phloem region, thus a root which has small cells there has also a pericycle of small cells.

The mature root is almost invariably limited on the external surface by the endodermis. This layer of cells has walls which are slightly thickened although not so obviously as those of the pericycle. It has arisen from the original endodermis normally by radial division of its cells. (G.straminea Fig.41b). Each separate cell is slightly elongated radially and is quite rich in protoplasm. The cells are in groups, each group having been derived from a single original endodermal cell.

Correlated with the difference in cell size of the outer tissues of the root there is also a slight difference in the cell size and shape in the endodermis. In the small celled type the endodermal cells are a little smaller although not markedly so, and they are

also slightly rounder in shape, particularly on the tangential walls. (G.Farreri Fig.45). The large celled type, where the cells are often oblong in outline, gives the impression that the endodermal cells have been dividing more rapidly and have not had the opportunity to resume the original oval shape of the endodermal cells or even to approach it.

The only abnormality visible in the endodermis is that in some cases the endodermal cells instead of dividing always in a radial direction have also sometimes divided at random (G.Loderi Fig.46). Of the six species which possess this feature G.angulosa, G.gracilipes, G.Loderi, G.tianschanica, G.cachemirica and G.sino-ornata, the last two show it only as an exceptional feature but in the first four it occurs frequently.

The endodermis however may not be the limiting layer as several of the oldest roots show pericyclic cork formation. In a group consisting of G.asclepiadea, G.cruciata, G.Makinoi, G.purpurea, G.scabra var Buergeri and G.tianachanica the first ring of cells of the pericycle next the endodermis becomes meristematic and acting as a phellogen gives rise to cells on its outer edge. The cells produced are oblong in shape and contain a fair amount of protoplasm. Later their cell walls become lignified and as a result the endodermis is shed (G.purpurea Fig.47a).

In a second group containing G.dahurica, G.Fetisowi, G.Kurroo and G.Veitchiorum a different process takes place. Here, no cork cambium is formed but a considerable number of thick-walled cells belonging to the pericycle and the outer region of the phloem and parenchyme collapse and their walls form a layer of dead tissue (G.Fetisowi Fig.47b). In none is the layer immediately within the endodermis affected but the disintegration certainly works out to the ring of cells next it.

Two other species fall to be mentioned here. The first is G.cachemirica in which cork cells are formed from a regular phellogen in some roots, while in other cases the dead tissue is formed by cells collapsing. Thus both types occur in one species.

In the other species, G.crassicaulis (Fig.48) several complications arise. In places a phellogen seems to develop immediately under the endodermis and gives rise to two or three rows of cells, the outermost being rich in protoplasm and having the external walls slightly lignified. At other places in the same section the cells of the pericycle collapse and dead tissue is thus formed; often the first layer of cells under the endodermis is untouched. Then immediately under these collapsed cells is a ring of cells rich in protoplasm with the walls next the collapsed cells slightly lignified. This layer

connects up with the similar layer mentioned above as being derived from the phellogen. The only difference between them is that those cells which occur under the collapsed tissue show a distinct tendency to divide in any direction instead of regularly and also the phellogen from which they have been derived is often obscure.

To the question of the disintegration of the outer tissues little importance beyond what has already been given can be attached. In those roots where the cells of the phloem and pericycle are small the outer tissues usually remain in good condition until a fairly late stage of development has been reached, while in the type where the cells of the phloem and parenchyme are larger the outer tissues may disappear very early (G.angustifolia Fig.2) or they may persist until later.

Two unusual points arise in connection with the outer tissues. Firstly there is the division of the cells of the hypodermis. This condition is fairly common and occurs frequently in the large cell type of root but not in the small type. What normally takes place is that each hypodermal cell is cut in two by a thin radial wall (G.Parryi Fig.49). G.asclepiadea (Fig.50) however goes a step further and here several radial walls appear in each cell making the hypodermis closely resemble the endodermis in type.

The second point is the division of the cortical cells. This was only found to any extent occurring in the root, of G.crassicaulis (Fig.51). The cells of the cortex appear to have divided in all directions and some are becoming lignified.

There seems to be no vital reason for the divisions occurring in these outer tissues except in that by dividing somewhat they will by that means retain their position round the growing stele longer than would be otherwise possible.

With regard to the question of the large and small celled types of roots their characteristics may be summarised as follows:-

Group A. Small-celled Type. e.g. G.sino-ornata (Fig.33b).

1. Little or no development of secondary xylem.
2. Amount of phloem always much greater than xylem.
3. Phloem, parenchyme and pericycle consist of small cells.
4. Walls of phloem equal in thickness to those of parenchyme.
5. Endodermis of rounded cells, indicating slow division.
6. External tissues retained in good condition until late stage.

Group B. Large-celled Type. e.g. G.Makinoi (Fig.33a).

1. Good or very good development of secondary xylem.
2. Amount of phloem sometimes greater than but usually equal to xylem.
3. Phloem, parenchyme and pericycle consist of large cells.

4. Walls of phloem are thicker than those of parenchyme.

5. Endodermal cells radially elongated, indicating quicker division.

6. External tissues usually shed early.

It should be noted however that these types are not of specific value as while the roots fall into one or other of the types the roots from one species may also show both types.

In five species roots of only the small-celled type were found. This group comprises:-

G.hexaphylla

G.hexaphylla X Farreri

G.Farreri

G.ornata

G.Macaulayi

The remainder of the species with four exceptions are in the large-celled group.

Two of the exceptions, G.verna and G.sino-ornata may have roots showing either of the two types while the third, G.prolata seems to commence by producing phloem and parenchyme of uniform thickness but when once the secondary thickening is well developed the later formed phloem then appears to be thicker walled than the parenchyme. The fourth exception, G.Veitchiorum differs from the small-celled type only in the possession of distinct secondary wood.

Briefly summarising, the main features of the roots are:-

1. The stele is normally diarch but may occasionally be triarch or tetrarch.

2. The secondary wood may be well lignified or mixed to varying degrees with parencyhme.

3. The secondary phloem is always in little groups embedded in a fair quantity of parenchyme. Two cases of interxylary phloem were found.

4. The pericycle starts as a single layer but later divides and often becomes thickened.

5. Cork formation is pericyclic.

6. The endodermis usually divides radially but occasionally tangentially.

7. The cortex may or may not include a fungus.

8. A distinct hypodermis is present in the young roots.

9. The epidermis produces no root hairs.

10. The outer tissues are shed.

Conclusion.

It will be seen that from the roots there is little information of value to the systematist to be obtained. While these roots show several interesting features anatomically the characters cannot be rigidly used to group the species. This occurs where two types of root anatomy are found in one species. This lack of differentiation between the species or groups of species may be due to the fact that the root, as such, possesses a comparatively uniform environment, the soil. Another factor influencing the root development may be the fact that as a genus, Gentiana is comparatively recent and there the roots may not have had the required time to diverge in structure to any marked extent.

GENTIANA - THE LEAF.

Introduction.

When the roots failed to reveal anything of systematic importance from their anatomy, it was decided to investigate the leaves.

In shape and size the leaves vary considerably, G.Farreri being at one end of the scale with very small needle-like leaves and G.asclepiadea at the other with very long and broad leaves. All degrees are to be found between these two. Again, the mid-rib may be very obvious as in G.Fetisowi or entirely lacking as in G.hexaphylla. Further some of the leaves are comparatively thick for their size, e.g. G.sino-ornata, while others are extremely thin, e.g. G.purpurea. It was hoped therefore that these variations in the external morphology would find a reflection in the anatomy.

The Young Leaf (in bud).

On the outside, the leaf is bounded by the epidermis. (G.Andrewsii Fig.52) Most of the cells of the epidermis are square in shape but those which lie on the under surface of the mid-rib are more columnar. The majority of the epidermal cells have a clearly distinguishable nucleus while the cells limiting the lamina have considerably more protoplasm than those limiting the mid-rib, owing to the fact that differentiation is not so far advanced in the lamina. The cells forming the main mass of the leaf are parenchymatous in nature, well supplied with protoplasm and possessing obvious nuclei.

In the mid-rib there are a considerable number of small air-spaces these being rather more apparent towards the under surface. The vascular bundle in the midrib which is naturally the most important one in the leaf is fairly well developed, especially in the phloem region. So far only two or three xylem vessels are visible and these are towards the upper side of the vascular bundle. The phloem however is quite plentiful and already the bundle shows signs of becoming bicollateral. The cells of the phloem are rich in contents and angular in shape. Lying between the xylem and the phloem are actively dividing cells which will form the cambium. To the left of the vascular bundle is a small area, rich in

protoplasm, which is evidently differentiating to form a side vein, while well out in the lamina are the beginnings of a vein represented by two xylem vessels. The remainder of the lamina consists of parenchyme which has not as yet differentiated to give the mesophyll. No stomata are so far visible.

The Young Leaf (bud opened).

In the next stage the tissues are definitely more differentiated (G.Andrewsii Fig.53). Towards the lower half of the mid-rib the parenchyme cells have increased considerably in size and as a consequence of this the intercellular spaces have grown larger. The vein in the mid-rib has also extended. Several xylem vessels are present and more are developing while the phloem is now quite obviously present on both sides of the xylem giving a bicollateral bundle.

The lamina is also developing but relatively slowly. Towards the outer end of each half of the lamina there is quite a large, well-formed vein but the remainder of the veins consist mainly of groups of cells rich in protoplasm which are not yet differentiated.

The mesophyll however is not now so uniform in structure. Towards the lower surface, the cells have increased considerably in size and intercellular spaces are definitely appearing. As the upper surface is approached the cells decrease in size

until these immediately under the epidermis are only about one quarter of the size of those in the lower half of the leaf. They are however definitely rich in protoplasm and will later give rise to the palisade mesophyll.

The epidermal cells on both the upper and lower surfaces are fairly uniform in shape except on the lower surface of the mid-rib, where they are larger and are elongated at right angles to the surface. There is still no sign of stomata interrupting the epidermal layer.

The Mature Leaf.

In the old leaf the tissues appear to be fully formed (G.Andrewsii Fig.54). The mid-rib is well-developed with the main vein placed slightly above the centre. Between the vein and the upper epidermis are a few rounded parenchyme cells with small intercellular spaces, while towards the lower surface the cells are again parenchymatous but are considerably larger. This increase in size also applies to the intercellular spaces. The vein is well formed possessing a fair amount of xylem and this is being added to by a cambium which is present below the xylem. On the lower side of the cambium lies the phloem, the presence of large nuclei in the companion cells making them conspicuous.

There is also an area of phloem on the upper side of the xylem thus completing the bicollateral bundle. There is no obvious bundle sheath but there are a few small parenchymatous cells surrounding the bundle.

The lamina has by now differentiated. Towards the lower half of the leaf is the spongy mesophyll, composed of irregular cells forming an exceedingly loose tissue. They are fairly rich in protoplasm and nucleated. The palisade tissue consists of one layer of cells immediately under the upper epidermis. Quite a large number of its cells possess an unusual feature. On the upper surface of the cell there is a U-shaped indentation which gives a slightly cordate shape to the cell. Where this occurs the nucleus tends to lie just at the base of the U or slightly below it, giving a characteristic appearance to the palisade layer.

Between the palisade layer and the veins in the lamina there is usually another layer of cells - the collecting cells. This layer however is not complete and is best seen just above the veins. The veins themselves are collateral and are surrounded by a bundle sheath.

In this leaf the upper and lower epidermis seem to possess little cuticle, their cells being thin walled and variable in size. The lower surface is now interrupted by the presence of stomata which

have developed. The stomata are quite normal and constant in shape throughout the species.

At the edge of the lamina the epidermal cells increase a little in size, their walls become thicker and their shape is more pointed.

This is the general type of development although there are several minor differences in the end result.

An attempt was made to group the species according to the structure of their leaves. The main points considered were:-

1. Structure of main vascular bundle.
2. Position of Stomata.
3. Presence of a projection at mid-rib.
4. Structure of palisade mesophyll.
5. Amount of cuticle present.
6. Construction of edge of lamina.
7. Size of epidermal cells.

Of these seven characters the first three with the fourth subsidiary proved to be the most dependable. The others were too variable and not sufficiently clean cut.

The result was that the species could be divided into four groups. These will now be described taking a typical example in each group and mentioning any variation from it.

Group A.

Group A.

Type - G.Andrewsii (Fig.54).

G.Andrewsii which has just been described is a typical member of this group, some of the others differing slightly.

G.Makinoi. In this case the palisade cells are slightly more elongated and no indentations are present. (These indentations are peculiar to G.Andrewsii). Cuticle is present on both surfaces to a small extent, there being a little more on the lower than on the upper.

G.scabra var.Buergeri has also a palisade layer whose cells are more elongated than those of G.Andrewsii. The upper epidermis (Fig.58) is a little different as some of the cells tend to be pear-shaped.

G.phlogifolia possesses some cuticle on both surfaces. In this leaf and the three following the palisade tissue does not give way to parenchyme above the main vascular bundle but is continuous.

G.Pneumonanthe has cuticle present.

G.cruciata possesses palisade cells more elongated than any of the others.

G.cephalantha (Fig.59) does not possess a well differentiated mesophyll. Here the palisade appears to be formed of two layers of cells, these cells being only very slightly elongated. The spongy mesophyll

is composed of fairly round cells and is not so loosely packed as in the other species mentioned.

G.asclepiadea (Fig.60) differs mainly in its shape. It has a much thinner lamina and this makes the mid-rib appear more prominent. Also two of the subsidiary veins on each half of the lamina are quite large and possess a structure similar to the mid-rib thus giving the leaf a characteristic shape. The structure of the lamina resembles that of G.cephalantha only the spongy mesophyll is slightly looser.

Group B.

Type - G.Veitchiorum (Fig.55).

This is a fairly small leaf but a comparatively thick one. There is a median vein which is larger than the others but there is no structure indicative of a mid-rib, that is, there is no projection on the lower surface and the palisade and spongy mesophyll do not give way to parenchyma at the centre of the leaf but are continued right across the lamina without a break.

All the vascular bundles including the main one are typical collateral bundles. In the main vein however there is a slight development of cambium. All the veins possess a good bundle sheath whose cells are circular in shape.

The mesophyll in the leaf is clearly differentiated into palisade and spongy tissue. The palisade tissue is about two cells in depth, these cells

being elongated and fairly compact. Immediately below the palisade, the cells of the mesophyll begin to branch and this reaches its greatest development in the lower half of the leaf forming there a very loose tissue. The cells of the upper epidermis are larger and possess more cuticle than those of the lower epidermis. Stomata occur on both surfaces. The guard cells of the stomata are smaller than the other epidermal cells and they possess no cuticle.

The leaf does not taper off to any extent at the edge of lamina but the epidermal cells in that position have a thicker layer of cuticle. The palisade tissue is continued well round the edge of the leaf.

G.hexaphyll X Farreri differs from the type only in that the palisade cells are not quite so elongated.

G.hexaphylla is also very close to the type, differing in that the palisade cells are less elongated and also the cuticle is not so thick on the upper surface.

G.sino-ornata is similar to G.hexaphylla.

G.Macaulayi is also similar to G.hexaphylla except that a few cells of parenchyme are present between the main vein and the lower epidermis.

Group C.

Type - G.crassicaulis (Fig.56).

This is a large leaf as are the majority of

those included in this group. The mid-rib is prominent and contains the main vascular bundle which is typically bicollateral. The parenchyme cells below the vein are loosely packed leaving a considerable number of air spaces. This is even more noticeable between the vein and the upper surface for in this region the cells are so loosely packed that they form single rows surrounding the air spaces.

The epidermal cells are fairly regular in shape, those of the upper surface being the larger. However the cells of both surfaces increase a little in size at the mid-rib. There is only a small amount of cuticle present but it increases slightly as the lamina tapers towards the edge. The stomata are present on both surfaces.

The mesophyll is divided as usual into the two regions and in this species the palisade is one cell thick, its cells being elongated. In most of the species in this group the palisade is two cells thick. The spongy mesophyll is quite typical.

G.Grombczewskii is a larger leaf than G.crassicaulis. In the mid-rib region lying beside the main vein are two smaller ones, one on each side and having the same bicollateral structure. The palisade layer is two cells thick but otherwise the structure resembles G.crassicaulis.

G.Fetisowi is structurally similar to

G.Grombczewskii but the palisade is occasionally three cells thick.

G.Kurroo and G.straminea are also typical and have a palisade two or three cells thick.

G.dahurica has a palisade also two cells in thickness. In this species moreover, the cells of the upper and lower epidermis are more irregular in shape and size and also they have more cuticle than is present in the majority of the species in this group. (Fig.61).

G.gracilipes is similar to G.dahurica but the epidermal cells are not so irregular and their cuticle is not so prominent. This leaf is smaller than the others.

G.tianschanica has also a smaller leaf and in this case the palisade is not replaced by parenchyme on the upper side of the mid-rib except for one layer which form a bundle sheath for the vein.

G.sceptrum resembles closely G.crassicaulis except that it does not possess the large air-spaces above the vein in the mid-rib.

G.verna, G.setigera and G.Newberryi are much alike. In size they fall between G.tianschanica and the larger type represented by G.crassicaulis. The palisade tissue which is not very well developed is two cells in thickness. In G.verna and G.setigera the palisade tissue is continuous along the upper half

of the leaf. G.Newberryi has a little extra cuticle.

G.acaulis and G.angustifolia differ from G.Newberryi only in that the palisade which is still indefinite is now three cells in thickness.

G.Kochiana and G.purpurea have larger and thinner leaves which taper more towards the edges. The palisade cells could scarcely be said to be elongated and indeed differ little from the cells of the remainder of the mesophyll, but can be identified because they are more closely packed.

Group D.

Type - G.Loderi (Fig.57)

The leaves of this group are medium in size and G.Loderi is no exception. The mid-rib is prominent forming a good projection on the under surface, the main vein being collateral. Between the vein and the lower epidermis there is the normal development of parenchyma and air-spaces while above it the parenchyma is partly replaced by palisade.

The epidermal cells vary slightly in size those of the upper epidermis being the larger. A little cuticle is present thickening towards the edge of the lamina which is comparatively rounded, its epidermal cells being quite large. Stomata are present on both surfaces.

The mesophyll is normal, the spongy mesophyll being composed of loosely packed cells while

the palisade is three cells thick, these being closely packed and elongated.

G.cachemirica agrees very closely with G.Loderi except that the palisade tissue is two cells thick.

G.gilvostriata has palisade tissue continuous along the upper surface, unbroken by parenchyma at the mid-rib and also the cuticle is thicker.

The table (Fig.58) gives the species indicating their characteristics.

Owing to the fact that the character of the palisade varies considerably and thus the difficulty arises of deciding just exactly when a definite palisade layer gives way to an indefinite one, the species were grouped using the character of the palisade as a feature within the Group and not as one governing it. The groups were as follows:-

Group A.

Bicollateral bundles: Stomata on under surface: Mid-rib with projection.

(1) One layer of definite palisade -

G.cruciata, G.phlogifolia, G.Makinoi, G.scabra var.Buergeri, G.Pneumonanthe, G.Andrewsii.

(2) Two layers of indefinite palisade -

G.cephalantha, G.asclepiadea

Group B.

Collateral bundles: Stomata on both surfaces: No projection at mid-rib.

(1) Two layers of definite palisade -

G.Farreri, G.Veitchiorum, G.sino-ornata, G.Macaulayi, G.hexaphylla, G.hexaphylla X Farreri.

(2) One to two layers of definite palisade -

G.ornata.

(3) One layer of definite palisade -

G.prolata.

Group C.

Bicollateral bundles: Stomata on both surfaces: Mid-rib with projection.

(1) Two layers of definite palisade -

G.gracilipes, G.tianschanica, G.Kurroo, G.straminea, G.dahurica, G.Fetisowi, G.Grombczewskii, G.macrophylla, G.trichotoma.

(2) One layer of definite palisade -

G.crassicaulis, G.sceptrum.

(3) Two layers of indefinite palisade -

G.verna, G.setigera.

(4) More than two layers of indefinite palisade -

G.acaulis, G.Kochiana, G.Newberryi, G.angustifolia

(5) Very indefinite palisade -

G.purpurea.

Group D.

Collateral bundles: Stomata on both surfaces: Mid-rib with projection.

(1) Two layers of definite palisade -

G.Loderi, G.cachemirica, G.gilvostriata, G.septemfida, G.stragulata.

(2) Two layers of indefinite palisade -

G.depressa.

A few of the species show a slight variation from the Group into which they have been placed.

G.prolata, G.macrophylla, and G.septemfida differ from their respective Groups in that they have only a few stomata on the upper surface of the leaf, but as otherwise they fit well it was considered impracticable to transfer them elsewhere.

G.stragulata is a species in which it is difficult to say whether the mid-rib projects or not. However as the lower half shows a definite mid-rib structure it has been placed in Group D.

G.ornata shows a different combination of characters from the others. It differs on three points from Group C, on two points from Groups A and D, and on one from Group B. Therefore it is possibly best placed in Group B although it has stomata only on the under surface.

G.Farreri (Fig.62) might be mentioned here as being unusual in that the palisade tissue is present under both surfaces.

Discussion.

When the species were formed into groups as described above it was thought it would be interesting

to compare these groups with those arrived at by the systematist. The most recent monograph of the genus is that by Kusnezow (2) where he has divided the genus into ten sections. In the following tables the systematic section of each species is indicated.

Group A.

Species.	Sections.
G.cruciata	Aptera
G.Phlogifolia	"
G.Makinoi	Pneumonanthe
G.scabra var.Buergeri	"
G.Pneumonanthe	"
G.Andrewsii	"
G.asclepiadea	"
G.cephalantha	Frigida

Group B.

Species.	Sections.
G.Farreri	Frigida
G.Veitchiorum	"
G.sino-ornata	"
G.Macaulayi	"
G.hexaphylla	"
G.hexaphylla X Farreri	"
G.ornata	"
G.prolata	"

Group C.

Species	Sections.
G.gracilipes	Aptera

G.tianschanica	Aptera
G.Kurroo	"
G.straminea	"
G.dahurica	"
G.Fetisowi	"
G.Grombczewskii	"
G.macrophylla	"
G.crassicaulis	"
G.trichotoma	Frigida
G.sceptrum	Pneumonanthe
G.setigera	"
G.Newberryi	"
G.verna	Cyclostigma
G.acaulis	Thylacites
G.angustifolia	"
G.Kochiana	"
G.purpurea	Coelanthe

Group D.

Species.	Sections.
G.septemfida	Pneumonanthe
G.stragulata	Frigida
G.gilvostriata	"
G.Loderi	Isomeria
G.cachemirica	"
G.depressa	"

The only really striking feature which emerges when the groups are compared with the systematic sections is that Group B all belong to the

section Frigida. However all the members of the Section Frigida examined do not fall into this Group. Thus G.cephalantha (Group A) is kept out of Group B because in its leaf it possesses bicollateral bundles and stomata only on the lower surface. G.trichotoma (Group C) differs on one point - it possesses bicollateral bundles, while G.stragulata and G.gilvostriata from Group D are separated by the possession of a midrib structure.

In Group A five of the species belong to the Pneumonanthe Section. Of this section three species are in Group C, while G.septemfida is in Group D.

In Group C nine of the species belong to the Section Aptera while the remainder of the species are found in various other Sections. Two of the species from this Section (Aptera), G.cruciata and G.phlogifolia are in Group A as they have stomata on the lower surface only.

In Group C are all three species of the Section Thylacites examined.

The only other noticeable feature is that the Isomeria Section species come together in Group D.

Conclusion.

From the evidence just given it would seem that there is no obvious correlation to be made between the Groups as formed during this investigation of the anatomy of the species and the Sections given

by their systematic relationships.

There is, however, one feature which is apparent and that is, the confirmation of the close relationship of the species given in Group B where all belong to the Section Frigida. Further proof of this was seen in the roots where all the species possessed roots of the small-celled type. These six species and two hybrids appear to form a group closely related by anatomical features as well as by systematic characters. Apart from these it would appear that the anatomical features in the genus have not developed parallel with its systematic characters.

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